CORRELATION BETWEEN PYOCIN-SENSITIVITY AND 2-AMINO SUGAR COMPOSITION OF *PSEUDOMONAS AERUGINOSA*

Nachiko SUZUKI
Institute of Medical Science, University of Tokyo, Takanawa, Tokyo, Japan

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1. Introduction

During the course of studies on 2-amino sugars in ten *Pseudomonas aeruginosa* strains, we have detected quinovosamine in only four strains, P2, P4, P14 and P15 [1]. These four strains are sensitive to pyocin produced by strain PI-III (designated as pyocin A) [2]. The bacteriocin sensitivity depends upon the presence of a specific receptor on the cell surface [3] and bacteriocin receptors have been observed in the lipopolysaccharide (LPS), the cytoplasmic membrane and the protein. Yet there has been no conclusive evidence that a specific residue such as quinovosamine plays an important role in pyocin sensitivity. Their relationship is therefore of interest. Quinovosamine is a constituent of the LPS of strain P14 [1], but it requires confirmation that quinovosamine is absent in other components of the cell.

This report demonstrates there is a correlation between the presence of quinovosamine and the sensitivity to pyocin A (or pyocin S produced by strain P16), and that quinovosamine is located in the side chain of the LPS.

2. Materials and methods

Two resistant mutants, P14PyR^r-2 and -3, of *P. aeruginosa* P14 against pyocin R produced by strain P15 were supplied by M. Kageyama. We derived a spontaneous revertant from strain P14PyR^r-3, which reverted to the sensitivity to pyocin R and was named P14M. The other twenty *P. aeruginosa* strains were selected from the collection of the IID. The bacteria were grown and harvested as described previously [1,4].

Assay for the pyocin sensitivity was carried out by the pyocin typing method of Darrell and Wahba [5] using neutral broth agar as the typing medium. Strains PI-III and P16 were used as a pyocin A and a pyocin S pyocinogenic strain, respectively. Strain PI-III has a temperate phage which acts on strain P14, while strain P16 has not. The pyocin receptor activity was estimated by inactivating activity of the sample for pyocin. As pyocin we used lysate, which was sterilized by chloroform and diluted twenty fold with broth, of over night culture of strain P16.

The LPS was isolated by the method of Westphal et al. [6], digested with ribonuclease A (Sigma) and Nagarse (protease) [7], and purified by zone electrophoresis (starch block, 1.5 × 7 × 40 cm; with 0.05 M borate buffer, pH 8.8, at 20 mA for 40 hr). Test on serological specificity was carried out by the precipitin reaction as previously described [7]. Fragmentation of the LPS was performed according to Schmidt et al. [8], except that after the remove of lipid A the hydrolysate (0.1 M HCl, 100°C, 30 min) was neutralized with 1 M NaOH and then fractionated on Sephadex G-50 by using water as eluant.

2-Amino sugar was chromatographed on Dowex 50 HW-X8 with 0.33 M HCl after hydrolysis (4 M HCl, 100°C, 4 hr) of the sample and estimated by the Boas modification of the Elson-Morgan reaction using galactosamine as a standard as previously described [1,4]. Reducing power was estimated by the method of Somogyi as previously described [4].

3. Results and discussion

Table 1 summarizes the 2-amino sugar composition and the pyocin sensitivity of twenty-two *P. aeruginosa* strains. All strains have glucosamine, galactosamine, muramic acid and two unidentified compounds (U-1, U-2). In addition to these principal components quinovosamine and fucosamine were detected in many strains. The breakdown of 2-amino sugars corresponds to figures previously published [1]. The strains could be classified into six groups dependent upon the characteristic patterns of 2-amino sugar composition. Pyocins A and S acted on all strains which had quinovosamine. If we assume that the pyocin sensitivity is related to the presence of quinovosamine, the resistant mutant should lose quinovosamine. The analytical data on two mutants of strain P14 supported this hypothesis; P14PyR^r-2 was resistant to pyocins A and S and lacked both quinovosamine and fucosamine, while P14M was sensitive to pyocins A and S and had them though in smaller amount than the parent strain P14.

The site of existence of quinovosamine in the cell was examined by check of the 2-amino sugar content of fractions obtained during the preparation of the LPS of strain P14. The LPS was extracted twice with 45% aqueous phenol from bacteria (11.6 g dry weight; total 2-amino sugar, 4.6%). The aqueous phase of the first extract contained 56% of total 2-amino sugar of the cell, and that of the second extract, 31%. The aqueous phases were digested by the enzymes and then dialysed. A recovery of 92% of total 2-amino sugar of aqueous phases was obtained in inner fluid. After concentration and dialysis against buffer the inner fluid was subjected to zone electrophoresis. Fractions from a single peak of 2-amino sugar of elution pattern were pooled, dialysed against water and then lyophilized (yield 970 mg; total 2-amino sugar, 43.8%). The LPS reacted in the antisera against strain P14 (reciprocal

Table 1
2-Amino sugar composition and pyocin sensitivity of *Pseudomonas aeruginosa* strains

Strains	U-1	U-2	Glucos-amine	Galactos-amine*	Quinovos-amine	Fucos-amine	Pattern	*Pyocins* A	S
P4	5.0	11.2	49.5	27.5	6.8	0	I	+	+
P9	3.1	11.1	52.7	25.5	7.7	0	I	+	+
P15	3.4	10.8	45.2	33.2	7.5	0	I	+	+
P20	4.8	10.4	53.9	23.6	7.3	0	I	+	+
P2	1.4	6.5	26.0	33.1	21.7	11.4	II	+	+
P5	5.7	6.2	28.1	33.8	19.1	7.2	II	+	+
P6	1.0	6.5	21.7	32.6	23.8	14.5	II	+	+
P10	0.5	5.7	20.8	33.2	23.5	16.4	II	+	+
P11	0.4	6.7	20.1	33.9	23.8	14.1	II	+	+
P14	1.9	4.8	21.4	32.8	25.4	13.7	II	+	+
P7	1.7	7.2	31.0	14.1	0	45.7	III	–	–
P13	5.0	6.1	21.9	18.6	0	48.5	III	–	–
P16	5.4	6.1	18.0	15.6	0	55.0	III	–	–
P19	0.9	5.9	22.6	17.7	0	52.9	III	–	–
N10	6.0	5.6	20.0	17.3	0	51.2	III	–	–
Pl-III	1.7	11.9	41.5	37.8	0	7.1	IV	–	–
P3	7.6	10.0	44.5	33.9	0	4.0	IV	–	–
P8	5.6	12.2	38.7	31.3	0	12.2	IV	–	–
P12	5.3	11.3	45.0	23.2	0	15.2	IV	–	–
P17	6.6	9.2	44.3	34.5	0	5.5	IV	–	–
P14PyR^r-2	5.4	9.6	52.8	32.2	0	0	V	–	–
P14M	2.6	12.3	48.1	29.4	4.1	3.5	VI	+	+

* contained muramic acid.
Values given are related to total content of 2-amino sugars detected as 100. Symbols: +, growth inhibition of strain by pyocin; –, no growth inhibition.

Table 2
Analyses of the LPS of strain P14 and its components

	Dry cells	LPS	F-A	F-B	Lipid A
Yield	100	8.4	3.4 – 4.2	1.7–2.5	1.7–2.1
U-1	0.08	0	0	0	0
U-2	0.20	0.9	0	0	1.8
Glucosamine	0.89	3.2	0	0	12.8
Galactosamine	1.37	16.2	28.8 (28.6)	12.1	0.9
Quinovosamine	1.06	12.6	24.0 (29.5)	1.6	0
Fucosamine	0.57	7.0	11.2 (12.8)	0	0
Unknown 2-amino sugar (U-7)	0.31	–	7.0 (–)	0	0
Ammonia			(9.8)		
Carbohydrate (as glucose)	–	9.3	6.2	26.5	–
Methylpentose (as rhamnose)	–	3.8	6.2	5.5	–
Heptose (as D-manno-heptulose)	–	0.8	0	2.7	–
KDO	–	–	0	3.1	–
Glycine	–	–	0.07	–	–
Alanine	–	–	0.10	–	–
Glutamic acid	–	–	0.26	–	–

2-Amino sugar was estimated as described in the text. Values in parenthese were obtained by the ninhydrin method using a JLC-5AH Autoanalyzer after hydrolysis (4 M HCl, 100°C, 4 hr) of the sample. Carbohydrate was estimated by the phenol/sulfuric acid method [9], methylpentose by the sulfuric acid/cysteine reaction [10], heptose by the Osborn modification [11] of the sulfuric acid/cysteine reaction, 2-keto-3-deoxyoctonic acid, KDO, by the tiobarbituric acid method [12] and amino acid by autoanalysis after hydrolysis (6 M HCl, 105°C, 22 hr). Yields are expressed as percentages of the dry cells and other values, of the sample by weight. –, not done.

titer was 64 000). Analytical data are given in table 2. The recovery of total 2-amino sugar amounted to about 80% of total 2-amino sugar of the dry cell; of glucosamine to 30%, of galactosamine to 95%, of quinovosamine to 99% and of fucosamine to 102%. These results indicate that quinovosamine exists only in the LPS.

The LPS consists of three different regions [13]; the lipid A, the core and the side chain. The lipid A contains glucosamine [14,15] and the core, galactosamine [13,16]. However, little was known about the nature of the side chain and any information had not been available on the site of existence of quinovosamine in the LPS. Therefore, we attempted to prepare the side chain of the strain P14. Fig. 1 shows the results of fractionation of the degraded polysaccharides.

Fraction A, which was previously filtrated on a Sephadex G-200 column (1.3 X 22 cm), and Fraction B were purified by refractionation on Sephadex G-50 using pyridine acetate solution as the eluant [8], and designated F-A and -B, respectively. Analytical data

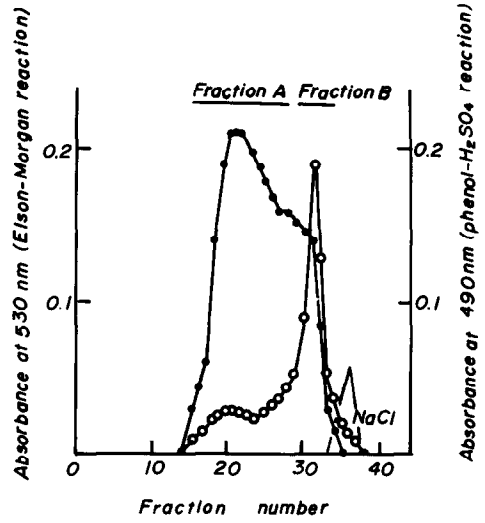

Fig. 1. Fractionation on Sephadex G-50 (1.2 X 94 cm) of the degraded polysaccharides prepared from the LPS (65 mg) of strain P14. Fractions (2.4 ml) were collected. Aliquots (0.1 ml) were analysed for carbohydrate [9] (o–o–o) and for 2-amino sugar (•–•–•). Fractions were pooled as indicated by horizontal bars and lyophilized.

are given in table 2. The spectrum of products of F-A obtained by the sulfuric acid/cysteine reaction indicated the presence of methylpentose but not hexose or heptose. However, this spectrum could be also explained by the fucosamine present [4]. Moreover, when F-A was hydrolysed (1 M HCl, 100°C) and the increase of reducing power and the liberation of 2-amino sugar were estimated, both values (as galactosamine) displayed the same time course (maximum value, 67%, after 4 hr). These facts suggest that methylpentose is not present in F-A. Thus, characterization of all F-A is still incomplete, but F-A may be regarded as the side chain as consisting of galactosamine, quinovosamine, fucosamine and an unknown 2-amino sugar (U-7), suggesting the possibility of the presence of repeating unit. F-B contained all the characteristic components of the core plus a small amount of quinovosamine. The presence of quinovosamine in F-B indicates that F-B is contaminated by a degraded portion of F-A rich in quinovosamine, because the core has no quinovosamine [16]. Consequently, it is concluded that quinovosamine is in the side chain. Strain P14PyR^r-2 might be a R mutant, and strain P14M, a semirough mutant.

Table 3 shows that the LPS of strain P14 acts as a receptor for pyocin S, while the LPS of strain N10, which has no quinovosamine, F-A and F-B do not. Although the side chain alone has no receptor activity, the analytical data of strains P14PyR^r-2 and P14M (table 1) suggests that small amounts of the side chain are necessary for the LPS to function as a receptor of pyocin.

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Table 3
Inactivation activity against pyocin S

Samples	μg wt/ml of reaction mixture	Indicator cells/ml	Survival cells/ml	Inactivation %
None	–	strain P14	6.5×10^5	0
LPS of strain P14	0.25	1.3×10^7	2.9×10^6	18
	0.5		4.5×10^6	31
	2.5		8.0×10^6	60
	5.0		1.0×10^7	76
None	–	strain P14	1.5×10^4	0
LPS of strain P14	3.0	8.5×10^6	5.8×10^6	68
LPS of strain N10	245		1.5×10^4	0
None	–	strain P15	6.7×10^5	0
LPS of strain P14	3.0	8.5×10^6	7.8×10^6	91
None	–	strain P14	1.4×10^6	0
F-A	375	6.2×10^6	1.6×10^6	4
F-B	175		1.4×10^6	0

Broth solution (0.25 ml) of a sample was mixed with 0.25 ml of pyocin S. The mixture was incubated at 37°C for 30 min. To the mixture an equal volume of a fresh broth culture of indicator strain was added and incubated at 37°C for 20 min. Survival was estimated by counting colonies.

References

[1] Suzuki, N., Suzuki, A. and Fukasawa, K. (1970) J. Japan. Biochem. Soc. 42, 130–134.
[2] Homma, J. Y. and Suzuki, N. (1966) Ann. N.Y. Acad. Sci. 133, 508–526.
[3] Jacob, F. (1954) Ann. Inst. Pasteur 86, 149–160.
[4] Suzuki, N. (1969) J. Japan. Biochem. Soc. 41, 769–776.
[5] Darrell, J. H. and Wahba, A. H. (1964) J. Clin. Path. 17, 236–242.
[6] Westphal, O., Lüderitz, O. and Bister, F. (1952) Z. Naturforschg. Pt. 7b, 148–155.
[7] Homma, J. Y. and Suzuki, N. (1964) J. Bacteriol. 87, 630–640.
[8] Schmidt, G., Jann, B. and Jann, K. (1969) European J. Biochem. 10, 501–510.
[9] Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A. and Smith, F. (1956) Anal. Chem. 28, 350–356.
[10] Dische, Z. and Shettles, L. B. (1948) J. Biol. Chem. 175, 595–603.
[11] Osborn, M. J. (1963) Proc. Natl. Acad. Sci. 50, 499–506.
[12] Weissbach, A. and Hurwitz, J. (1959) J. Biol. Chem. 234, 705–709.
[13] Fenson, A. H. and Meadow, P. M. (1970) FEBS Lett. 9, 81–84.
[14] Fenson, A. H. and Gray, G. W. (1969) Biochem. J. 114, 185–196.
[15] Chester, I. R., Gray, G. W. and Wilkinson, S. G. (1972) Biochem. J. 126, 395–407.
[16] Ikeda, K. and Egami, F. (1973) J. Gen. Appl. Microbiol. 19, 115–128.